June 26, 2006

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
For

Use of Petrifilm and PetriScan® for Research Applications
SOP Number: EQ-09-00

U.S. Environmental Protection Agency

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SOP Number: EQ-09-00

Date Revised: 06-21-06

Initiated By: _____ Date: ___/___/___

Print Name: _____

Technical Review: _____ Date: ___/___/___

Print Name: _____
Technical Staff

QA Review: _____ Date: ___/___/___

Print Name: _____
QA Officer

Approved By: _____ Date: ___/___/___

Print Name: _____
Branch Chief

Effective Date: ___/___/___

Controlled Copy No.: _____

Withdrawn By: _____ Date: ___/___/___

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1.0 SCOPE AND APPLICATION:

1.1 The ability to recover and quantify microorganisms used in disinfectant and sporicidal efficacy testing is critical to evaluate the efficacy of a product. This SOP describes the use of 3M Petrifilm™ Aerobic Count (AC) Plate and PetriScan® with *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* for research purposes.

2.0 DEFINITIONS:

2.1 CFU = Colony Forming Units

2.2 TNTC = Too Numerous to Count

2.3 AOAC = AOAC International

2.4 Audit View = A secure database that records and displays user, date, time, and edited data when records are changed.

2.5 Flag = A code notation assigned by PetriScan® indicating film edits or changes in other plate characteristics.

2.6 Pouch = One pouch contains 50 Petrifilm.

2.7 Plate = Petrifilm plate

3.0 HEALTH AND SAFETY:

3.1 All manipulations of the test organisms are required to be performed in accordance with biosafety practices stipulated in SOP MB-01, Lab Biosafety.

3.2 Counting the Petrifilm using the scanner will be performed on the bench-top.

4.0 CAUTIONS:

4.1 Hold the pipette perpendicular to the film to dispense the sample.

4.2 Immediately after spotting the sample on the Petrifilm, the sample must be pressed with the spreader to distribute the sample.

4.3 The sample must be pressed straight down – do not slide the spreader across the film.

4.4 The Petrifilm must be allowed to rest undisturbed for one minute after spreading to allow the gel to form.

4.5 Petrifilm Aerobic Count plate components are decontaminated though not sterilized.

5.0 INTERFERENCES:

5.1 Do not use plates that show discoloration.

5.2 Do not use diluents containing citrate, bisulfite or thiosulfate with Petrifilm plates; these compounds can inhibit growth.

5.3 Since the colonies grow suspended in a gel matrix, the application of substantial pressure to the film will cause the colonies to shift; thus the application of pressure to the film after spreading should be avoided.

5.4 High concentrations of colonies on the Petrifilm plates will cause the entire growth area to become red or pink. Occasionally, on overcrowded plates, the center may lack visible colonies, but many small colonies can be seen on the edges. When any of these occurs, do not count the plates using the scanner – record the results as TNTC.

5.5 Some organisms can liquefy the gel, allowing them to spread out and obscure the presence of other colonies.

5.6 Dust particles may be picked up by the PetriScan® and counted as colonies – analysts must be sure to evaluate each film after the program performs its **Auto Count** function.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Petrifilm™ Aerobic Count Plate

7.2 PetriScan®

7.3 Computer in B202 loaded with PetriScan® software

7.4 Hewlett-Packard Scanjet 8200

7.5 Incubator at 37±1°C or another temperature suitable for growth of the target organism

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 The PetriScan® has been validated (refer to the PetriScan® User Guide, page 29, section 3.9). This data will be archived.

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Store unopened Petrifilm plate pouches refrigerated or frozen at temperatures ≤8°C. Just prior to use, allow unopened pouches to come to room temperature before opening.

9.2 Return unused plates to pouch. Seal by folding the end of the pouch over and taping shut.

9.3 To prevent exposure to moisture, close pouch tightly before placing back into the refrigerator.

9.4 Resealed pouches may be stored for no longer than one month.

10.0 PROCEDURE AND ANALYSIS:

10.1 Petrifilm

10.1.1 Mark the plate identification information within the top ¾ inch portion of the film. Barcode labels may also be placed in this area.

10.1.2 Prepare dilutions to be plated using appropriate sterile diluents (e.g., PBDW, Luria-Bertani broth, letheen broth, or distilled water).

10.1.3 For plating, use dilutions that will yield between 0-300 CFU per plate.

10.1.4 Place the Petrifilm plate on a flat, level surface.

10.1.5 Lift the top film and hold the pipette perpendicular to dispense the appropriate amount (800, 900 or 1000 µL, depending upon the total volume of the dilution) of sample suspension onto the center of the bottom film.

10.1.6 Drop the top film down onto the sample.

10.1.7 Place the plastic spreader with the recessed side down on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire Petrifilm plate growth area before the gel is formed.

10.1.8 Remove the spreader and leave the plate undisturbed for at least one minute to permit the gel to form.

10.1.9 Incubate plates in a horizontal position with the clear side up in stacks of no more than 20 plates at 37±1°C for 24-48 hours (depending on the organism being evaluated). An incubation time of 48 hours is necessary for *Pseudomonas aeruginosa* as well as for microorganisms damaged by exposure to disinfectants or sporicides.

10.1.10 Petrifilm can be counted using a standard colony counter or PetriScan®.

10.1.11 Count all red colonies regardless of size or intensity.

10.1.12 Confirmation

10.1.12.1 Colonies may be isolated for further identification by lifting up the top film and picking up growth from the gel. These colonies can then be gram stained or plated.

10.2 PetriScan®

10.2.1 Turn on the computer and double click the PetriScan® icon on the desktop. The sign on dialog box will be displayed. Enter the appropriate user name and password.

10.2.2 PetriScan® data is stored and maintained in database files. After signing on, the analyst must either create a new database or open an existing database. To create a new database, refer to the PetriScan® User Guide, page 13, section 2.2.1. To open an existing database, refer to the PetriScan® User Guide, page 14, section 2.2.2.

10.2.3 To count colonies using the PetriScan®, lift the scanner lid and place 1-4 Petrifilms face-down within the cutouts on the alignment grid.

10.2.3.1 Do not count plates that contain any of the following conditions:

- High concentrations of colonies on the Petrifilm plates that cause the entire growth area to become red or pink.
- Overcrowded plates where the center lacks visible colonies, but the edges contain colonies.
- Excessive spreader growth (>50% of the film area).

10.2.3.2 If liquefied gel interferes with counting, an estimated count

should be made by counting the unaffected areas.

10.2.4 Close the lid and click **Scan**. When the scan is complete, the images will be displayed in the grid view. (Attachment 1)

10.2.5 After scanning, enter in an **ID** and **Dilution** factor (if applicable) for each film to be counted.

10.2.6 Click **Select All**, or select each film individually by placing a ✓ mark in the box in the lower left-hand corner of the film.

10.2.7 Click **Auto Count**. PetriScan® will mark each counted colony with a green circle and display the number of counted colonies and the CFU/mL or CFU/gm. The CFU/mL or CFU/gm represents the calculated colony forming units per milliliter or gram of the original sample, taking into account the dilution factor.

To count film manually, place a ✓ mark in the box in the lower left-hand corner of the film to be counted and click **Manual Count**. As necessary, add or delete colonies using the left and right mouse buttons, respectively. Click **Exit**. Select **Yes** when asked if the data should be saved.

10.2.8 After automatic counting, click **Save Data**. Films marked with ✓ marks will be saved as JPEG (.jpg) image files, and data for the selected films will be saved to the open database and displayed in the Data View. The grid will be cleared of images/data and will be ready for the next scan.

NOTE: Data is not saved to the database until **Save Data** is clicked. If the **Scan** button is clicked for the next set of Petrifilm to be scanned before saving data, the images and data will be lost.

10.2.9 It is recommended that after scanning and saving the data, the analyst should review each film to be sure it was counted correctly. To review each scanned film, double-click on an individual entry to bring up the image of the scanned film. Observe the film to make sure all colonies have been counted (e.g., encircled in blue) and to make sure that all spots that were counted are colonies (occasionally a dust particle will be counted as a colony).

It is also recommended that the **ID** and **Dilution Factor** entered by the analyst be checked against the ID and Dilution Factor noted on the label of the Petrifilm.

11.0 DATA ANALYSIS/CALCULATIONS:

11.1 To edit colonies (this option is only available after saving), double-click on the row listed in the Data View.

11.1.1 To remove marked colonies, click with the right mouse button on the green circles. The circles will turn red to show that they will not be used in the count.

11.1.2 To add colonies that were not marked as counted, click with the left mouse button. A blue circle will mark the colony, indicating that it will be added to the count. To exclude a newly-marked circle, simply click on it again with the right mouse button.

11.1.3 Click **Exit** and select **Yes** when asked if the data should be saved.

11.1.4 Enter the reason for editing the data and click **OK**. The saved data will replace the row in the Data View, and a flag will be assigned (refer to the PetriScan® User Guide, page 27, section 3.6.2 for information regarding flags). The previous data, flag notations, and reason for change will be archived to Audit View.

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 PetriScan® software creates an FDA/GLP-compliant database, which contains every saved plate, including its image and the settings used in its analysis. This allows images to be recalled and re-analyzed at a later date.

12.2 PetriScan® tracks changes made to the database, in compliance with Code of Federal Regulations, Title 21, Part 11, Electronic Records; Electronic Signatures, and Code of Federal Regulations, Title 21, Part 58, Good Laboratory Practice for Non-Clinical Laboratory Studies. These changes can be seen in the Audit View by clicking the **Audit View** button (refer to the PetriScan® User Guide, page 35, section 4.4 for information regarding Audit View).

13.0 QUALITY CONTROL:

13.1 Quality control procedures described in the PetriScan® User Guide, pages 28 and 29, section 3.8 will be used to verify the count accuracy and consistency at both low and high colony counts of the PetriScan® system once a month while the unit is in use.

13.1.1 The electronic data generated during these quality control procedures will be stored in a database entitled *Quality Control*. Once per year, the electronically archived quality control data will be saved as a Microsoft

Excel spreadsheet, printed and filed.

13.2 The sterility of each pouch will be checked.

13.2.1 Each pouch (per box) will be individually numbered 1-20 (there are twenty pouches per box of 1000 Petrifilm).

13.2.2 Upon opening a new pouch of Petrifilm, one film will be randomly selected per pouch, spotted with 1 mL of sterile DI water, and incubated at 37±1°C for 5-10 days. Results (e.g., growth or no growth) will be recorded on form 16.1.

13.2.3 The lack of red colonies will indicate a sterile film, while the presence of one or more red colonies will indicate contamination.

13.2.4 Contaminated film (e.g., the film within the pouch where the contamination was noted) will be discarded.

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 If any colonies are detected on the uninoculated films used for quality control procedures, visually inspect the film for defects or particles. If no particles are present, it may be necessary to clean the scanner (refer to the PetriScan® User Guide, page 41, section 5).

14.2 For further information involving problems encountered in operating the PetriScan®, refer to the PetriScan® User Guide, page 43, section 6 (Troubleshooting).

15.0 REFERENCES:

15.1 PetriScan® User Guide, Spiral Biotech, Inc., Rev3 110104.

15.2 Hewlett-Packard Scanjet 8200 Series Scanners User's Manual, Hewlett-Packard, 2003.

15.3 3M Petrifilm™ Aerobic Count Plate Pamphlet, 3M, 2004 (38-9018-1246-1).

16.0 FORMS AND DATA SHEETS:

16.1 Sterility Assessment Log
16.2 Attachment 1: Sample Film with Growth
16.3 Attachment 2: Sample Data Spreadsheet
16.4 Attachment 3: Sample Audit View

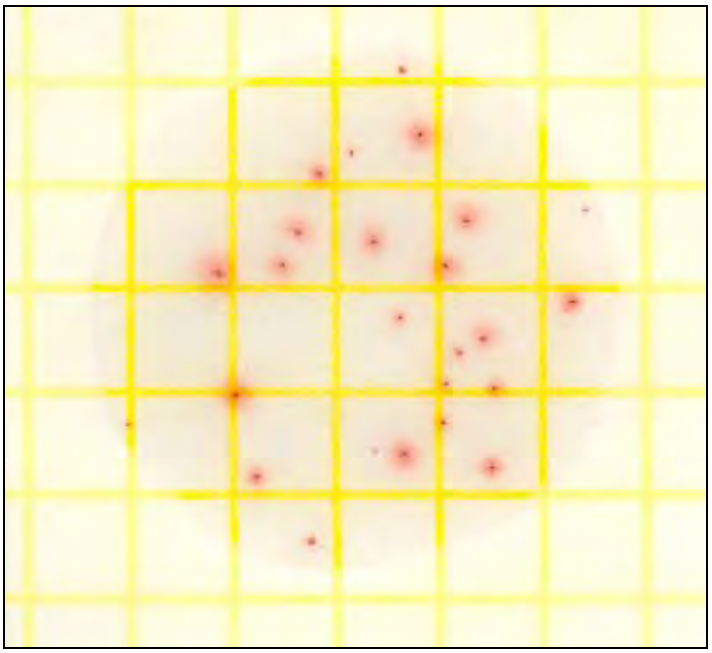
Petrifilm Sterility Assessment Log
OPP Microbiology Laboratory

Date/Initials	Pouch #	Control #	Petrifilm Lot #	Sterile DI Water Prep #	Incubation End Date/Initials	Was Growth Observed?*
						☐ Yes ☐ No
	2					☐ Yes ☐ No
	3					☐ Yes ☐ No
	4					☐ Yes ☐ No
1	5					☐ Yes ☐ No
	6					☐ Yes ☐ No
	7					☐ Yes ☐ No
	8					☐ Yes ☐ No
	9					☐ Yes ☐ No
	10					☐ Yes ☐ No
	11					☐ Yes ☐ No
	12					☐ Yes ☐ No
	13					☐ Yes ☐ No
	14					☐ Yes ☐ No
	15					☐ Yes ☐ No
	16					☐ Yes ☐ No
	17					☐ Yes ☐ No
	18					☐ Yes ☐ No
	19					☐ Yes ☐ No
	20					☐ Yes ☐ No

*The observation of no growth indicates a passing sterility assessment. If growth is observed, discard the contaminated film (e.g., all of the film within the pouch where the contamination was noted).

Attachment 1

Sample Film with Growth



Attachment 2

Sample Data Spreadsheet

Name	Experiment #1								
Title	QC								
Reference									
High Count Limit	300								
Low Count Limit	0								
Created by	MLB								
Plate #	**Plate ID**	**Dilution**	**Count**	**CFU/ml**	**Flag**	**Date**	**Time**	**User ID**	
1	Template 1	0	30	3.00E+01		3/17/2006	11:34:11 AM	MLB	
2	Template 2	0	202	2.02E+02		3/17/2006	11:34:11 AM	MLB	
3	Uninoculated 1	0	1	1.00E+00		3/17/2006	11:34:11 AM	MLB	
4	Uninoculated 2	0	0	0.00E+00		3/17/2006	11:34:12 AM	MLB	
5	Template 1	0	30	3.00E+01		3/17/2006	11:43:58 AM	MLB	
6	Template 2	0	202	2.02E+02		3/17/2006	11:43:59 AM	MLB	
7	Template 1	0	30	3.00E+01	ED	3/17/2006	11:43:59 AM	MLB	
8	Template 2	0	202	2.02E+02		3/17/2006	11:43:59 AM	MLB	

Attachment 3

Sample Audit View

Name	SAT vs. TSM (High Counts)								
Title	500 CFU Upper Limit								
Reference									
High Count Limit	500								
Low Count Limit	0								
Created by	MLB								
Plate #	**Plate ID**	**Dilution**	**Count**	**CFU/ml**	**Flag**	**Mod Date**	**Mod Time**	**Mod By**	**Reason**
500	6/042506, B, 10^0	0	52	5.20E+01		4/26/2006	2:31:48 PM	MLB	Additional colonies.
499	5/042506, B, 10^0	0	54	5.40E+01		4/26/2006	2:30:46 PM	MLB	Additional/extraneous colonies.
498	4/042506, B, 10^0	0	96	9.60E+01		4/26/2006	2:29:46 PM	MLB	Additional/extraneous colonies.
497	3/042506, C, 10^-2	0	5	5.00E+00		4/26/2006	2:27:40 PM	MLB	Extraneous colony.
496	3/042506, C, 10^-1	0	66	6.60E+01		4/26/2006	2:27:31 PM	MLB	Additional colonies.
495	2/042506, C, 10^-3	0	3	3.00E+00		4/26/2006	2:27:09 PM	MLB	Extraneous colonies.
494	2/042506, C, 10^-2	0	13	1.30E+01		4/26/2006	2:26:55 PM	MLB	Extraneous colonies.
493	2/042506, C, 10^-1	0	46	4.60E+01		4/26/2006	2:26:32 PM	MLB	Additional colonies.
492	1/042506, C, 10^-1	0	14	1.40E+01		4/26/2006	2:25:58 PM	MLB	Additional/extraneous colonies.
491	3/042506, B, 10^-4	0	5	5.00E+00		4/26/2006	2:25:42 PM	MLB	Extraneous colonies.
490	3/042506, B, 10^-3	0	78	7.80E+01		4/26/2006	2:25:22 PM	MLB	Additional/extraneous colonies.
489	3/042506, B, 10^-2	0	449	4.49E+02		4/26/2006	2:24:22 PM	MLB	Additional colonies.
488	2/042506, B, 10^-4	0	11	1.10E+01		4/26/2006	2:23:00 PM	MLB	Additional/extraneous colonies.
487	2/042506, B, 10^-3	0	53	5.30E+01		4/26/2006	2:22:42 PM	MLB	Additional/extraneous colonies.
486	2/042506, B, 10^-2	0	433	4.33E+02		4/26/2006	2:22:25 PM	MLB	Additional colonies.
485	1/042506, B, 10^-4	0	7	7.00E+00		4/26/2006	2:20:57 PM	MLB	Extraneous colony.
484	1/042506, B, 10^-3	0	59	5.90E+01		4/26/2006	2:20:44 PM	MLB	Additional/extraneous colonies.
483	1/042506, B, 10^-2	0	403	4.03E+02		4/26/2006	2:20:23 PM	MLB	Additional colonies.
482	3/042506, A, 10^-4	0	5	5.00E+00		4/26/2006	2:19:11 PM	MLB	Extraneous colonies.
480	3/042506, A, 10^-2	0	140	1.40E+02		4/26/2006	2:18:50 PM	MLB	Additional colonies.
479	2/042506, A, 10^-4	0	3	3.00E+00		4/26/2006	2:18:31 PM	MLB	Extraneous colonies.
478	2/042506, A, 10^-3	0	9	9.00E+00		4/26/2006	2:18:16 PM	MLB	Additional/extraneous colonies.
477	2/042506, A, 10^-2	0	99	9.90E+01		4/26/2006	2:18:00 PM	MLB	Additional/extraneous colonies.
476	1/042506, A, 10^-4	0	6	6.00E+00		4/26/2006	2:17:21 PM	MLB	Extraneous colonies.